Chapter 2

Age-dependent role of pre- and perinatal factors in interaction with genes on ADHD symptoms across adolescence

Djûke M. Brinksma
Pieter J. Hoekstra
Barbara van den Hoofdakker
Annelies De Bildt
Jan. K. Buitelaar
Catharina A. Hartman
& Andrea Dietrich

Journal of Psychiatric Research (2017), 90, 110-117

ABSTRACT

Background

Little is known about the effects of risk factors on Attention-Deficit/Hyperactivity Disorder (ADHD) symptoms over time. Here, we longitudinally studied the role of candidate genes, pre- and perinatal factors, and their interactions on ADHD symptoms between ages 10 and 18 years.

Methods

Subjects were part of the general population or clinic-referred cohort of the TRacking Adolescents' Individual Lives Survey (n = 1,667). At mean ages of 11.1 (T1), 13.4 (T2), and 16.2 years (T3), ADHD symptoms were assessed with the Child Behavior Checklist. Linear Mixed Models were used to examine the association of candidate genes (i.e., DRD4, *DRD2*, *5-HTTLPR*, *COMT*, and *MAOA*), pre- and perinatal factors (i.e., index measure of various pregnancy and delivery complications, maternal smoking, maternal drinking, and low birth weight), and their interactions with ADHD symptoms across adolescence.

Results

Pregnancy and delivery complications were associated with a higher level of ADHD symptoms across all time points, but with a significantly declining influence over time ($p = .006$). We found no main effects of the candidate genes on ADHD symptoms throughout adolescence. The simultaneous presence of the low activity *MAOA* genotype and low birth weight ($p < .001$) and of the *5-HTTLPR* LL-allele and respectively pregnancy and delivery complications ($p = .04$) and maternal smoking ($p = .04$) were associated with more ADHD symptoms particularly during early adolescence, and these influences significantly decreased over time.

Conclusions

Findings suggest an age-dependent role of gene-environment interactions on ADHD symptoms across adolescence.

INTRODUCTION

Attention-Deficit/Hyperactivity Disorder (ADHD) is a childhood-onset disorder with age-inappropriate symptoms of inattention and hyperactivity/impulsivity (American Psychiatric Association, 2013), which tend to decrease over time (Faraone et al., 2006), but with a highly variable long-term course (Van Lier et al., 2007). Although genetic (Faraone & Mick, 2010; Gizer et al., 2009), pre- and perinatal factors (most notably maternal smoking during pregnancy and low birth weight; Banerjee et al., 2007), and gene-environment (G×E) interactions (Neuman et al., 2007) have been implicated in the etiology of ADHD, their possibly changing effect over time on ADHD symptoms remains largely unknown. Recent research in twins indicated that inter-individual differences in the overall decline in ADHD symptoms could be explained by genetic and environmental influences that are largely distinct from those influencing the onset of symptoms (Pingault et al., 2015). This is consistent with the idea that the role of risk factors for ADHD may change over time (Thapar et al., 2007).

In contrast to the wealth of cross-sectional studies that investigated genes in association with ADHD (Faraone & Mick, 2010; Gizer et al., 2009; Brookes et al., 2006), few studies have examined the role of genetic factors during the course of ADHD symptoms. The *dopamine D4 receptor* gene (*DRD4*) 7-repeat was found to be associated with a more persistent course of ADHD symptom severity over time (Biederman et al., 2009; Langley et al., 2009), whereas the presence of the long version of the *serotonin transporte*r gene (*5-HTTLPR*) was not associated with the course of ADHD (Biederman et al., 2009).

It is reasonable to assume that pre- and perinatal factors have a long lasting role on ADHD symptoms, given that unfavorable prenatal conditions were linked to persistently high trajectories of ADHD symptoms from infancy to middle childhood (Galéra et al., 2011), and were associated with a diagnosis of ADHD, even up to 40 years after birth (Halmøy et al., 2012). However, no studies are available that examined the role of pre- and perinatal variables on ADHD symptom levels during the course of adolescence.

The aim of the current study was to investigate the association of a number of ADHD candidate genes (*DRD4*, *5-HTTLPR*, *dopamine D2 receptor* [*DRD2*], *catechol-O-methyl transferase* [*COMT*], and *monoamine oxidase A* [*MAOA*]), a set of pre- and perinatal factors (an index of various pregnancy and delivery complications, maternal smoking during pregnancy, maternal drinking during pregnancy, and low birth weight), and their interactions with changing levels of ADHD symptom from early to late adolescence.

METHODS

Study Sample

The present study contained 1,667 adolescents (92.0% Dutch descent) of whom genetic information was available and who participated in the first (T1; M_{age} = 11.09), second (T2; M_{age} = 13.37), and/or third (T3; M_{age} = 16.16) wave of the Tracking Adolescents' Individual Lives Survey (TRAILS). TRAILS consists of a general population cohort (n = 2,230 at T1) and a parallel clinic-referred cohort (n = 543 at T1; see Oldehinkel et al., 2015 and De Winter et al., 2005 for more sample characteristics). Children from the clinic-referred cohort had been referred to the Groningen university child and adolescent outpatient clinic at least once. The child's parents or legal guardian and adolescents (≥12 years) provided both written informed consent prior to each wave, whereas younger participants provided verbal assent. The TRAILS study was approved by the Central Committee on Research Involving Human Subjects (Dutch CCMO).

Procedure

At T1, T2, and T3 parents filled out a questionnaire on adolescent's ADHD symptoms. At T1, trained interviewers used the TRAILS Family History Interview (Ormel et al., 2005) to assess pre- and perinatal variables by interviewing one of the parents or guardians (preferably the mother, 95.6%). Blood or buccal cells were collected for DNA analysis (see further below).

Measures

ADHD symptoms. The DSM-IV-Oriented subscale Attention-Deficit/Hyperactivity Problems of the CBCL (Achenbach, 1991; Verhulst & Achenbach, 1995) consisting of 7 items was used as outcome measure of ADHD symptoms at all three waves. Items were scored by parents on a 3-point Likert-scale ranging from 0 = not true to 2 = very true or often true, over the past six months. For descriptive purposes, ASEBA cut-off scores (Achenbach, 1991) were used to categorize adolescents with clinical (>Percentile97 [P97]), subclinical (between P90-P97), and normal (<P90) ADHD symptom levels.

Pre- and perinatal factors. Based on Buschgens and colleagues (2009), we created an index score of the total number of 31 possible complications (observed range 0-12, mean = 1.83, SD = 2.10) related to pregnancy (e.g., physical, social, or psychological problems during pregnancy), delivery (e.g., breech presentation, Caesarean section), and neonatal hospitalization of the child (e.g., lack of oxygen, blood transfusion, jaundice) or the mother, as an overall score of a suboptimal pre- and perinatal environment. Three groups were created based on the number of events: 0 = no complications (35.9%), 1 = few complications (between 1 and 4 complications, 52.6%), and 2 = many complications (≥ 5 complications, 11.4%).

Maternal smoking at any time during pregnancy was categorized as 0 = nonsmokers (71.4%), 1 = mild smokers (daily use of 1-10 cigarettes or occasional smoking; 22.5%), and 2 = moderate smokers (daily use of ≥ 10 cigarettes; 6.1%, cut-off based on Maughan et al., 2004). Maternal alcohol use at any time during pregnancy was divided into 0 = nonusers (80.7%), 1 = mild drinkers (< 1 glass a week; 14.1%), and 2 = moderate drinkers (≥ 1 glass a week; 5.2%). A birth weight less than 2500 grams (standard clinical cut-off) was categorized as a low birth weight (4.2%). Correlations between the pre- and perinatal factors showed positive associations between maternal smoking and alcohol use during pregnancy ($r = .07$, $p < .001$), and between the pregnancy and delivery complication index and low birth weight ($r = .17$, $p < .001$).

Candidate Genes

We selected all ADHD neurotransmitter-related candidate genes from recent meta-analyses (Brookes et al., 2006; Gizer et al., 2009) that were available in TRAILS; unfortunately, the dopamine transporter gene (*DAT1*) was not available.

Genotyping. Genotyping of the length polymorphisms (LP) *DRD4, HTTLPR* including SNP rs25331 (A/G SNP in *L HTTLPR*), and *MAOA* was done at the Radboud University Nijmegen Medical Centre in Nijmegen, The Netherlands. The *DRD2* TaqIA (rs1800497), and the *COMT* val158met (rs4680) were genotyped by the Golden Gate Illumina BeadStation 500 platform (Illumina Inc., San Diego, CA) according to the manufacturers protocol. Genotyping procedures are documented elsewhere (Stavrakakis et al., 2013).

Genotype model. We considered the 7-repeat of *DRD4*, the A1 allele of *DRD2*, the long version of *5-HTTLPR*, the Val-allele of *COMT*, and the high activity alleles of *MAOA* as 'risk' alleles for a more persistent course of ADHD, based on the etiological literature. We used dominant models for *DRD4* and *COMT*, an additive model for the tri-allelic classification of *5-HTTLPR,* and a recessive model for *DRD2*. The functional status of heterozygous females is uncertain given that *MAOA* is X-linked. Based on previous findings (Reif et al., 2014), heterozygous females carrying at least one long allele (3.5, 4 or 5 repeats; Deckert et al., 1991) were categorized in the high transcription group. Table 1 shows the genotype distribution. For a description of genotyping methods see Supplementary Information 2.1.

Data Analysis

We applied Linear Mixed Models (LMMs) using data from T1, T2, and T3 to investigate the role of (1) five candidate genes in separate models (i.e., two-way interactions between the respective candidate gene and time), (2) one set of four pre- and perinatal factors simultaneously in one model to examine unique effects mutually adjusting for one another (i.e., all two-way interactions between pre- and perinatal variables and time), and (3) their G×E interaction per candidate gene in separate models (i.e., three-way

interactions of the respective candidate gene, all four pre- and perinatal factors, with time) on ADHD symptom levels across adolescence. LMMs allow for missing data at different waves, an important advantage for a longitudinal design (Kwok et al., 2008). Time was used as a proxy for the course of ADHD symptoms, with the overall sample mean age at T1 as the intercept (starting point) and change scores in individuals' age ($T1\text{-}T1_{(Mage)}$; $T2\text{-}T1_{(Mage)}$; $T3\text{-}T1_{(Mage)}$) as the slope (change). Sex and past-year ADHD medication (i.e., methylphenidate, dexamphetamine, and atomoxetine) use (0 = no use, 1 = use at any time in the preceding year) were included as covariates. As a priori analyses did not indicate an effect of population stratification in relation to the investigated candidate genes, there was no need to include principal genetic component scores as covariates into the final models or restrict analyses to subjects of Dutch ancestry. To adjust for the potential effects of covariates, we included all covariates×G, covariates×E, and covariates×time interaction terms in the G×E models (Keller, 2014). For readability purposes, beta values were multiplied by 1000 throughout text and tables. In case of a significant gene-environment correlation (*r*GE), the unstandardized residuals of the genotype, derived from linear regression, were included in the respective G×E analysis (Nederhof et al., 2012).

For interpretational purposes, the predicted ADHD symptom scores of significant two- or three way interactions were saved based on the estimated regression coefficients. Significant differences between slopes were tested using Generalized Estimating Equation (GEE) using an unstructured correlation matrix (Pepe & Anderson, 1994). Also, pairwise comparisons of mean ADHD symptom levels between the variations of the respective genotype and the different levels of pre- and perinatal variables for each of the waves (i.e., within and between) were conducted using General Linear Models. This was done to enhance insight into possible age-related differences. Analyses were performed using PASW Statistics 19. Restricted Maximum Likelihood estimation was used. A *p*-value of $< .05$ (two-sided) was considered to be statistically significant; the significance level corrected for multiple G×E comparisons would be 0.0025 (0.05/20) given the five investigated genes and four different pre- and perinatal factors.

RESULTS

Sample Descriptives

Table 1 presents sample characteristics and the genotypic distributions. ADHD symptom severity decreased in a similar pattern in both sexes, with a stronger decline from T1 to T2 than from T2 to T3. ADHD symptom levels in the clinical range were observed for 176 (10.6%) of the adolescents at T1, 118 (7.1%) at T2, and 71 (4.3%) at T3. Subclinical levels were found for 135 adolescents (8.1%) at T1, 137 (8.7%) at T2, and 126 (8.6%) at T3.

Table 1 *Sample characteristics*

Variable	*T1*	*T2*	*T3*
n	1667	1583	1458
Male gender	51.1%	50.6%	50.8%
Age in years, *M* (*SD*) [range]	11.1 (0.55) [10.0-12.6]	13.47 (0.61) [11.6-15.1]	16.2 (0.68) [14.4-18.4]
ADHD medication[a,b], *N* (%)	152 (9.1%)	174 (10.4%)***	145 (8.7%)***
♂/♀	15.1% / 2.8%***	11.4% / 9.4%	14.2% / 2.9%***
ADHD severity[c,d], *M* (*SD*)	0.67 (0.52)	0.53 (0.49)***	0.48 (0.45)***
♂/♀	0.78 (0.53) / 0.56 (0.49)***	0.62 (0.51) / 0.43 (0.45)***	0.56 (0.48) / 0.38 (0.40)***
Genotype distribution (n, %)			
DRD4 (7^- carriers; 7^+ carriers)	1059 (63.5%)		608 (36.5%)
DRD2 (GG; A-carriers)	1063 (63.8%)		604 (36.2%)
5-HTTLPR (SS; LS; LL)	419 (25.1%)	828 (49.8%)	420 (25.1%)
COMT (Met/Met; any Val-allele)	475 (28.5%)		1192 (71.5%)
MAOA (low activity; high activity)	426 (25.6%)		1241 (74.4%)

Note: ADHD =Attention-Deficit/Hyperactivity Disorder; DRD4=Dopamine D4 Receptor; DRD2=Dopamine D2 Receptor; 5-HTTLPR=Serotonin Transporter; COMT=Catechol-O-Methyl Transferase; MAOA=Monoamine oxidase A gene.

[a]Methylphenidate, dexamphetamine and atomoxetine past year use (1) or non-use (0).

[b]Significant differences with prior wave as well as between sexes by using χ^2-test, respectively.

[c]Significant differences with prior wave as well as between sexes by F-test, respectively.

[d]Based on the Child Behavior Checklis (CBCL; Achenbach, 1991), DSM-IV-oriented ADHD subscale (score range 0-2).

***p < .001

Candidate Genes During the Course of ADHD Symptoms

None of the candidate genes were related to ADHD symptoms over time (*p*-values .06 to .97), although the *DRD2* gene was marginally significant ($p = .06$). Subsequent pairwise comparisons showed that A-allele carriers had significantly lower ADHD symptom severity at T1, T2, and T3 (*p*-values < .001 to .006) compared to G-allele homozygotes of *DRD2*.

Pre- and Perinatal Factors During the Course of ADHD Symptoms

Of the four two-way interactions between pre- and perinatal factors and time, that of pregnancy and delivery complications was significantly related to ADHD symptoms across adolescence ($b = -8.10$, $p = .006$; see Information Supplementary Information 2.2 and 2.3). There was a similar, albeit marginally significant effect of maternal smoking during pregnancy ($b = -6.13$, $p = .06$). The slopes of the three levels of pregnancy and delivery complications (none, few, and many) differed significantly from each other (*p*-values <.001 to .04). Adolescents with many complications had significantly higher ADHD symptom levels at each wave compared to adolescents with few or no complications. Also, those with few complications had significantly higher ADHD symptom levels than those with no complications (all *p*-values < .001).

G×E Interactions During the Course of ADHD Symptoms

Because *DRD4* and low birth weight (r = -.05, $p < .05$), and *MAOA* and maternal drinking during pregnancy (r = .05, $p < .05$) showed significant *r*GEs, unstandardized residuals of *DRD4* and *MAOA* were used in the respective G×E analysis. The LMMs (Table 2) showed three significant three-way interactions related to ADHD symptoms across adolescence: *5-HTTLPR*×pregnancy and delivery complications×time (b = -9.08, p = .04), *5-HTTLPR*×maternal smoking during pregnancy×time (b = -9.82, p = .04), and *MAOA*×low birth weight×time (b = 85.72, $p < .001$). No significant G×E interactions were found for *DRD2*, *DRD4*, and *COMT*.

***5-HTTLPR* × index score of pregnancy and delivery complications.** Figure 1 (upper panel) shows that L-allele carriers had distinct ADHD symptom levels across adolescence depending on the number of pregnancy and delivery complications, with strongest effects for L-allele homozygotes. GEE analyses indicated that the slope of L-allele homozygotes with many pregnancy and delivery complications showed the steepest decline of ADHD symptoms from T1 to T3 compared to all other slopes (Δb = 14.57 to 29.74, p-values < .001 to .004), except for the slope of L-allele heterozygotes with many complications which was marginally significant (Δb = 10.00, p = .08). Moreover, the pairwise comparisons showed that L-allele homozygotes with many complications had the most severe ADHD symptoms compared to those with few or no complications, both within and across the allelic variation of the *5-HTTLPR*, not only at T1 (p-values < .001 to .008), but also T2 (p-values < .001 to .02), and T3 (p-values < .001 to .02). However, L-allele homozygotes with many complications did not differ compared to S-allele carriers with many pregnancy and delivery complications at T2 and T3 and a few other exceptions (p-values between .07 and .82). Thus, for L-allele homozygotes, larger differences in ADHD severity between adolescents with no, few, or many pregnancy and delivery complications were found in early than in late adolescence. This pattern was also found for L-allele heterozygotes, albeit to a lesser extent; also here, differences in ADHD symptom levels remained significantly different between L-allele heterozygotes with no, few, or many complications at all three waves (all p-values < .001).

Finally, the pairwise comparisons showed that S-allele homozygotes without pregnancy and delivery complications had less severe ADHD symptoms than those with few or many complications at all waves (all p-values ≤ .001). S-allele homozygotes with few or many complications also differed in ADHD symptom levels from each other at T2 (p = .006) and T3 (p = .03), but not at T1 (p = .12).

***5-HTTLPR* × maternal smoking during pregnancy.** Figure 1, lower panel shows the three-way interaction between *5-HTTLPR*×maternal smoking during pregnancy×time, similar to the results for pregnancy and delivery complications. Again, the significantly steepest decline of ADHD symptoms was found for L-allele homozygotes with many levels of maternal smoking (Δb = 25.58 to 46.97, p-values ≤ .001), with the highest ADHD

symptom levels compared to all other adolescents at T1 (*p*-values < .001 to .002). However, at T3, these moderately exposed L-allele homozygotes had similar ADHD symptom levels as moderately exposed adolescents carrying at least one S-allele of the *5-HTTLPR* genotype (*p*-values of .75 and .77). Thus, L-allele homozygotes exposed to severe levels of maternal smoking during pregnancy conveyed the greatest risk for the most severe ADHD symptoms in early but not late adolescence.

Table 2 *Three-way interactions between respectively the 5-HTTLPR and MAOA genes × pre- and perinatal factors × time during the course of ADHD symptoms across adolescence*[a]

	5-HTTLPR			*MAOA*		
Parameter	Estimate[b]	SE[b]	*p*	Estimate[b]	SE[b]	*p*
Intercept[c]	474.40	48.96	<.001	427.51	71.73	<.001
Time	-33.71	6.95	<.001	-27.11	9.40	.004
Genotype[d]	-53.47	38.74	.168	-8.75	76.43	.909
MSDP[e]	57.45	47.41	.226	64.29	69.43	.355
MDDP[e]	5.92	53.34	.912	-0.93	75.88	.990
PDCs[f]	45.24	45.62	.322	133.71	63.89	.037
LBW[g]	118.25	153.69	.442	277.86	230.65	.102
Genotype[d]*Time	8.30	5.28	.116	0.37	9.65	.969
MSDP[e]*Time	5.76	6.44	.371	0.27	7.95	.973
MDDP[e]*Time	-3.24	6.91	.639	-17.07	8.80	.053
PDCs[f]*Time	1.88	6.04	.756	-7.53	7.23	.301
LBW[g]*Time	2.64	1.96	.893	-78.09	24.92	.002
Genotype[d]*MSDP[e]*Time	-9.82	4.84	.043	-4.83	7.83	.537
Genotype[d]*MDDP[e]*Time	-1.55	4.75	.745	13.54	8.63	.117
Genotype[d]*PDCs[f]*Time	-9.08	4.31	.035	0.89	7.03	.899
Genotype[d]*LBW[g]*Time	-5.73	1.34	.669	85.72	24.32	<.001

Note, MSDP=Maternal smoking during pregnancy; MDDP=Maternal drinking during pregnancy; PDCs=Index of pregnancy and delivery complications; LBW=Low birth weight, see further Table 1.

[a]Adjusted for sex and ADHD medication

[b]Values multiplied by 1000 to increase readability

[c]Significant variability ($p < .001$) in intercept for ADHD symptoms for both 5-HTTLPR ($var(u_{0j})=197.53$, $\chi^2(1)=22.14$) and MAOA ($var(u_{0j})=198.14$, $\chi^2(1)=22.11$)

[d]5-HTTLPR coded as SS-carriers (0), LS-carriers (1), or LL-carriers (2); MAOA as low (0) or high activity (1)

[e]MSDP and MDDP coded as no (0), mild (1), or moderate (2)

.[f]PDCs coded as no (0), few (1), or many (2)

[g]LBW coded as yes (0) or no (1).

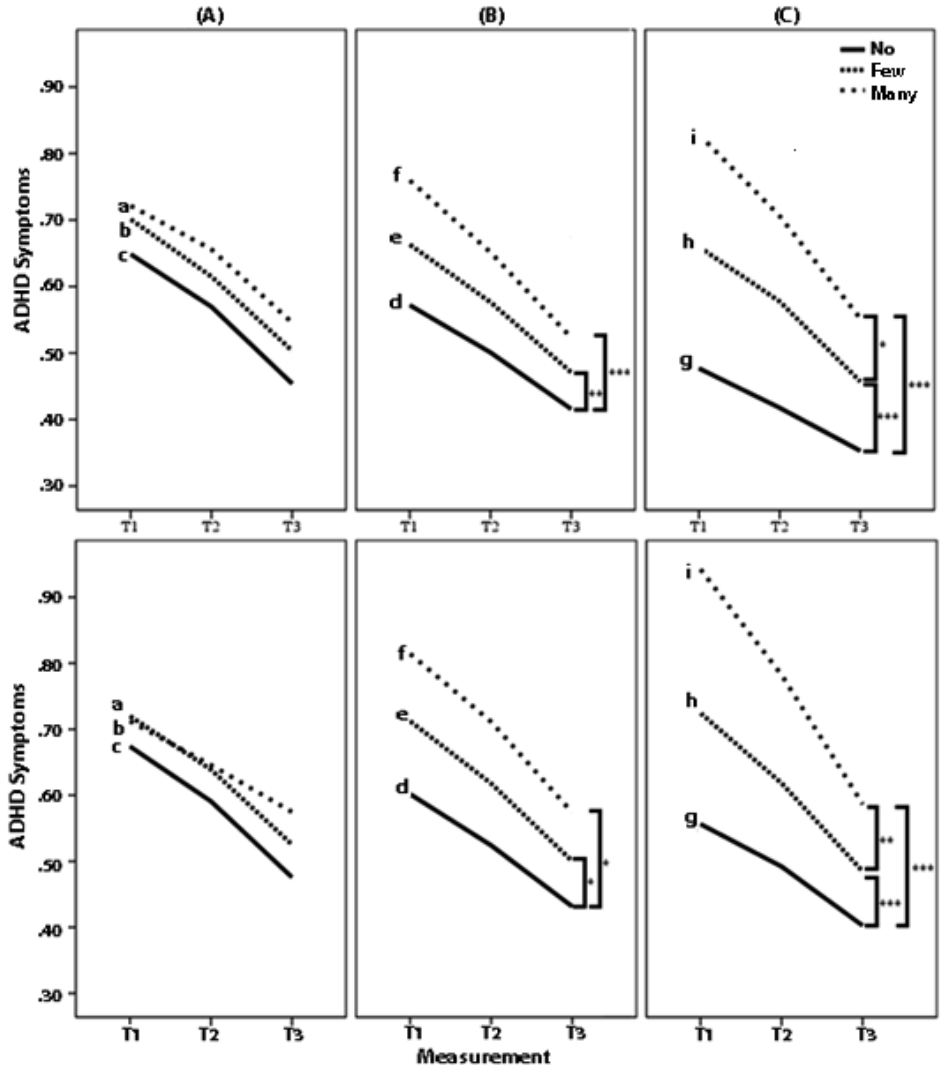

Figure 1. Attention-deficit/hyperactivity disorder (ADHD) symptom levels plotted for different levels of an index score pregnancy and delivery complications (UP, *upper panel)* and maternal smoking during pregnancy (LP, *lower panel*) separately for **(A)** S-allele homozygotes, **(B)** L-allele heterozygotes, and **(C)** L-allele homozygotes of the *5-HTTLPR* genotype across T1 (M = 11.1 yrs, T2 (M = 13.4 yrs), and T3 (M = 16.2 yrs). Significant slope differences: ***p < .001 (UP: bg [i.e., b differs from g, etc.], dh, di, df, eg, gh, gi; LP: ai, bi, ci, di, dh, gh, gi), ** p < .01 (UP: ad, ag, ai, bd, bi, ci, de, ei; LP: ag, bh, ch, eg, fg, fi, hi), and *p < .05 (UP: hi, LP: ad, ah, bf, cf, de, df).

***MAOA* × low birth weight.** Figure 2 indicates that adolescents with the low activity *MAOA* and low birth weight showed the steepest decline in ADHD symptom levels from T1 to T3 (Δb = 74.71 to 84.94, all p-values < .001) and the highest ADHD symptom levels at T1 compared to all other adolescents (all p-values < .001). Notably, ADHD symptom

levels at T3 of adolescents with low activity *MAOA* and a low birth weight approached those of adolescents without low birth weight ($p = .05$), and adolescents with high activity *MAOA* with and without low birth weight ($p < .001$). Interestingly, adolescents with the high activity *MAOA* without low birth weight had the least severe ADHD symptoms at all three waves compared to all other adolescents (all p-values < .001). Of note, given that *MAOA* is a sex-linked gene, we performed additional post-hoc exploration of results, which indicated similar patterns for boys and girls (due to small cell sizes for girls, statistics for only boys are presented in Supplementary Information 2.4).

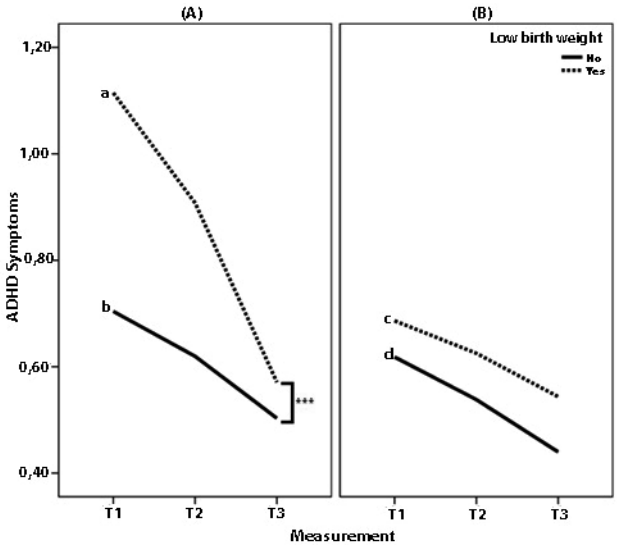

Figure 2. Attention-deficit/hyperactivity disorder (ADHD) symptom levels plotted for the presence and absence of a low birth weight, separately for the (**A**) low activity and (**B**) high activity *MAOA* genotype across T1 ($M = 11.1$ yrs), T2 ($M = 13.4$ yrs), and T3 ($M = 16.2$ yrs).
Significant slope differences: ***$p < .001$ (ab [i.e., a differs from b, etc.], ac, ad).

DISCUSSION

This study investigated the role of several ADHD candidate genes, pre- and perinatal adversities, and their interactions on ADHD symptom levels across three time points in a pooled population and clinic-referred sample of adolescents from age 10 to 18 years. Our results indicate age-dependency of a number of G×E interactions on ADHD symptom levels across adolescence; G×E interactions were primarily apparent in early adolescence and tended to level off over time. In line with the literature (Pingault et al., 2015), we observed a general decline of ADHD symptoms.

More specifically, we observed an age-dependent interaction of *MAOA* with birth weight. Adolescents with low activity *MAOA* and low birth weight appeared most at risk for elevated levels of ADHD symptoms during early adolescence. Similarly, age-dependent G×E interactions were found for *5-HTTLPR* with an index of pregnancy and delivery complications and maternal smoking, respectively. Results showed that L-allele carriers (particularly L-allele homozygotes) are more vulnerable to pre- and perinatal adversities than S-allele carriers particularly during early and middle adolescence. Taken together, these findings suggest that *MAOA* and *5-HTTLPR* genotypes moderate the influence of pre- and perinatal adversities on ADHD symptoms primarily during the earlier phases of adolescence, an association that diminishes as adolescents grow towards adulthood.

It should be noted that the results of this study are best considered as preliminary and are in need of replication given the paucity of similar studies on G×E interactions, a field that is often hampered by non-replication (Dick et al., 2015; Duncan & Keller, 2011). Only the G×E interaction of *MAOA* with low birth weight above the significance threshold corrected for multiple testing, hence, especially the findings regarding the *5-HTTLPR* genotype should be considered with caution.

An important finding was a distinct role of the low activity *MAOA* genotype in relation to a more unfavorable course of ADHD as opposed to the high activity *MAOA* genotype that has been linked to ADHD etiology (Gizer et al., 2009), thus providing support for the existence of factors that are specifically related to the course of ADHD symptoms as previously suggested (Pingault et al., 2015; Thapar et al., 2007). Interestingly, the high activity *MAOA* genotype appeared to play a protective role specifically in the absence of a low birth weight, as opposed to the low activity *MAOA* genotype.

The possible involvement of the long but not the short version of the *5-HTTLPR* interacting with pre- and perinatal adversities in relation to more severe ADHD symptoms is consistent with previous studies (Gizer et al., 2009). Yet, in a recent study S-allele carriers of the *5-HTTLPR* genotype were shown to be more sensitive to psychosocial stress in relation to ADHD symptoms (Van der Meer et al., 2014). The *5-HTTLPR* genotype may thus differentially interact with the type of environmental stressor. Underlying mechanisms of pre- and perinatal adversities are unclear but fetal hypoxia may be at play (Allen et al., 1998). However, we would like to stress that our suggested associations between the pre- and perinatal factors and ADHD symptom levels are no proof of causality (see Langley et al., 2012).

We did not find support for an independent role of candidate genes on ADHD symptom levels across adolescence, although there was a marginally significant effect of *DRD2* genotype. Negative findings of the *5-HTTLPR* genotype in relation to the course of ADHD symptoms have been previously reported (Biederman et al., 2009), although the *DRD4* 7-repeat was found to be associated with a more persistent course of ADHD in two studies (Biederman et al., 2009; Langley et al., 2009). Discrepant findings may be due to

methodological differences between the studies (e.g., age, sex, the use of continuous measures of ADHD symptoms versus clinical diagnosis, investigation of subtypes of ADHD, or study design). In addition, overall genetic influences as well as the impact of specific genes (independent from environmental factors) may vary across development, for example, being more evident during either childhood (Kuntsi et al., 2005) or young adulthood (Dick et al., 2006). However, we did find an age-dependent role of pregnancy and delivery complications across adolescence independent of genotype.

In sum, our results emphasize the importance of G×E interactions during the course of ADHD symptoms (Thapar et al., 2007), in line with previous evidence that a large proportion of the genetic variation is specific to the developmental course, not necessarily shared with the onset of symptoms (Pingault et al., 2015). Further, our findings corroborate the evidence that genetic influences may change over time (Chang et al., 2013), resulting in G×E interactions being more evident at some points during development than at others. Moreover, our study is consistent with the finding that prenatal adversities heighten the likelihood of persistently high ADHD symptom levels from infancy to middle childhood (Galéra et al., 2011), yet points to a declining influence during adolescence.

Strengths and Limitations

This prospective study provides novel evidence on the role of G×E interactions on ADHD symptoms within a large sample of adolescents in the transition into adulthood, an understudied area in ADHD research so far. Clearly, replication of our findings is needed. While retrospective recall of pre- and perinatal factors may have been a limitation of this study, retrospective recall of maternal smoking during pregnancy and of low birth weight have been found to be accurate (Rice et al., 2007). Moreover, we used a pooled sample of a population and clinic-referred cohort to increase power to detect significant G×E effects. However, this limits generalizability to pure population or clinical samples.

Conclusions

This study supports the notion that genetic factors in interaction with exposure to pre- and perinatal adversities have a changing role during the course of ADHD symptoms beyond childhood. Importantly, our findings suggest that the here reported G×E interactions on ADHD symptom severity decrease over time and may not carry over into adult age. This highlights the importance of taking developmental age into account when studying risk factors for ADHD, especially in cross-sectional studies with wide age ranges. Future studies are necessary to extend our preliminary findings to other age ranges. Particularly, investigating age-dependent effects of genetic and perinatal risk factors from the very onset of ADHD symptoms would be important up into adulthood. Such studies may also focus on epigenetic mechanisms.

REFERENCES

Achenbach, T. (1991). *Manual for the Child Behavior Checklist/4-18 and 1991 Profile.* Burlington, VT: University of Vermont Department of Psychiatry.

American Psychiatric Association (2013). *Diagnostic and statistical manual of mental health disorders: DSM-5 (5th ed.)*. Arlington, VA: American Psychiatric Publishing.

Allen, N.B., Lewinsohn, P.M., & Seeley, J.R. (1998). Prenatal and perinatal influences on risk for psychopathology in childhood and adolescence. *Development and. Psychopathology, 10*(3), 513-529.

Banerjee, T.D., Middleton, F., & Faraone, S.V. (2007). Environmental risk factors for Attention-Deficit Hyperactivity Disorder. *Acta Paediatrica, 96*(9), 1269-1274.

Biederman, J., Petty, C.R., Ten Haagen, K.S., Small, J., Doyle, A.E., Spencer, T., ... Faraone, S.V. (2009). Effect of candidate gene polymorphisms on the course of Attention Deficit Hyperactivity Disorder. *Psychiatry Research, 170*(2-3), 199-203.

Brookes, K., Xu X., Chen, W., Zhou, K., Neale, B., Lowe, N., ... Johansson, L. (2006). The analysis of 51 genes in DSM-IV combined type Attention Deficit Hyperactivity Disorder: Association signals in DRD4, DAT1 and 16 other genes. *Molecular Psychiatry, 11*(10), 934-953.

Buschgens, C.J.M., Swinkels, S.H.N., Van Aken, M.A.G., Ormel, J., Verhulst, F.C., & Buitelaar, J.K. (2009). Externalizing behaviors in preadolescents: Familial risk to externalizing behaviors, prenatal and perinatal risks, and their interactions. *European Child and Adolescent Psychiatry*, *18*(2), 65-74.

Chang, Z., Lichtenstein, P., Asherson, P.J., Larsson, H. (2013) Developmental twin study of attention problems: High heritabilities throughout development. *JAMA Psychiatry*, *70*(3), 311-318.

De Winter, A.F., Oldehinkel, A.J., Veenstra, R., Agnes, J., Verhulst, F.C., & Ormel, J. (2005). Evaluation of non-response bias in mental health determinants and outcomes in a large in a large of non-response bias in mental health determinants and outcomes sample. *European Journal of Epidemiology, 20*(2), 173-181.

Deckert, J., Catalano, M., Syagailo, Y.V., Bosi, M., Okladnova, O., Di Bella, D., ... Lesch K.P. (1999). Excess of high activity monoamine oxidase A gene promoter alleles in female patients with panic disorder. *Human Molecular Genetics, 8*(4), 621-624.

Dick, D.M., Agrawal, A., Keller, M.C., Adkins, A., Aliev, F., Monroe, S., & Sher, K.J. (2015). Candidate gene-environment interaction research: reflections and recommendations. Perspectives on Psychological Science, 10(1), 37-59.

Dick, D.M., Bierut, L., Hinrichs, A., Fox, L., Bucholz, K.K., Kramer, J., ... Foroud, T., (2006). The role of gabra2 in risk for conduct disorder and alcohol and drug dependence across developmental stages. *Behavior Genetics, 36*(4), 577-590.

Duncan, L.E., & Keller, M.C. (2011). A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. *American Journal of Psychiatry, 168*(10), 1041-1049.

Faraone, S.V., Biederman, J., & Mick, E. (2006). The age-dependent decline of Attention Deficit Hyperactivity Disorder: A meta-analysis of follow-up studies. *Psychological Medicine, 36*, 159-165.

Faraone, S.V., Mick, E. (2010). Molecular genetics of Attention Deficit Hyperactivity Disorder. *Psychiatric Clinics of North America*., *33*(1), 159-180.

Gizer, I.R., Ficks, C., & Waldman, I.D. (2009). Candidate gene studies of ADHD: A meta-analytic review. *Human Genetics, 126*(1), 51-90.

Galéra, C., Côté, S.M., Bouvard, M.P., Pingault, J., Melchior, M., Michel, G., Boivin, M., & Tramblay, R.E. (2011). Early risk factors for hyperactivity-impulsivity and inattention trajectories from age 17 months to 8 years. *Archives of General Psychiatry, 68*(12), 1267-1275.

Halmøy, A., Klungsøyr, K., Skjærven, R., & Haavik, J. (2012). Pre- and perinatal risk factors in adults with Attention-Deficit/Hyperactivity Disorder. *Biological Psychiatry, 71*(5), 474-481.

Keller, M.C. (2014). Gene × environment interaction studies have not properly controlled for confounders: The problem and the (simple) solution. *Biological Psychiatry, 75*(1), 18-24.

Kuntsi, J., Rijsdijk, A., Ronald, A., Asherson, P., & Plomin, R., (2005). Genetic influences on the stability of Attention-Deficit/Hyperactivity Disorder symptoms from early to middle childhood. *Biological Psychiatry, 57*(6), 647-654.

Kwok, O., Underhill, A.T., Berry, J.W., Luo, W., Elliott, T.R., & Yoon, M. (2008). Analyzing longitudinal data with multilevel models: An example with individuals living with lower extremity intra-articular fractures. *Rehabilitation Psychology, 53*(3), 370-386.

Langley, K., Fowler, T.A, Grady, D.L., Moyzis, R.K., Holmans, P.A, van den Bree, M.B.M., Owen, M.J., O'Donovan, M.C., & Thapar, A. (2009). Molecular genetic contribution to the developmental course of Attention-Deficit Hyperactivity Disorder. *European Child & Adolescent Psychiatry, 18*(1), 26-32.

Langley, K., Heron, J., Smith, G.D., & Thapar, A. (2012). Maternal and paternal smoking during pregnancy and risk of ADHD symptoms in offspring: testing for intrauterine effects. *American Journal of Epidemiology, 176*(3), 261-268.

Maughan, B., Taylor, A., Caspi, A., & Moffitt, T.E. (2004). Prenatal smoking and early childhood conduct problems. *Archives of General Psychiatry, 61*(8), 836-843.

Nederhof, E., Belsky, J., Ormel, J., & Oldehinkel, A.J. (2012). Effects of divorce on Dutch boys' and girls' externalizing behavior in Gene × Environment perspective: Diathesis stress or differential susceptibility in the Dutch Tracking Adolescents' Individual Lives Survey study? *Development and Psychopathology, 24*(3), 929-939.

Neuman, R.J., Lobos, E., Reich, W., Henderson, C.A., Sun, L., Todd, R.D.(2007). Prenatal smoking exposure and dopaminergic genotypes to cause a severe ADHD subtype. *Biological Psychiatry, 61*(12), 1320-1328.

Oldehinkel, A.J., Rosmalen, J.G.M., Buitelaar, J.K., Hoek, H.W., Ormel, J., Raven, D., ... Hartman, C.A. (2015). Cohort profile update: The Tracking Adolescents' Individual Live Survey (TRAILS). *International Journal of Epidemiology, 44*(1), 76-76n.

Ormel, J., Oldehinkel, A.J., Ferdinand, R.F., Hartman, C.A., De Winter, A.F., Veenstra, R., Vollebergh, W.A., Minderaa, R.B., Buitelaar, J.K., & Verhulst, F.C. (2005). Internalizing and externalizing problems in adolescence: General and dimension-specific effects of familial loadings and preadolescent temperament traits. *Psychological Medicine, 35*(12), 1825-1835.

Pepe, M.S., & Anderson, G.L. (1994). A cautionary note on inference for marginal regression models with longitudinal data and general correlated response data. *Communications in Statistics, 23*, 939-951.

Pingault, J., Viding, E., Galéra, C., Greven, C.U., Zhen, Y., Plomin, R., & Rijsdijk, F. (2015). Genetic and environmental influences on the developmental course of Attention-Deficit/Hyperactivity Disorder symptoms from childhood to adolescence. *JAMA Psychiatry, 72*(7), 651-658.

Reif, A., Richter, J., Straube, B., Höfler, M., Lueken, U., Gloster, A. T., ... Deckert, J. (2014). MAOA and mechanisms of panic disorder revisited: From bench to molecular psychotherapy. *Molecular Psychiatry, 19*(1), 122-128.

Rice, F., Lewis, A., Harold, G., van den Bree, M., Boivin, J., Hay, D.F., Owen, M.J., & Thapar, A. (2007). Agreement between maternal report and antenatal records for a range of pre- and perinatal factors: The influence of maternal and child characteristics. *Early Human Development, 83*(8), 497-504.

Stavrakakis, N., Oldehinkel, A.J., Nederhof, E., Oude Voshaar, R.C., Verhulst, F.C., Ormel, J., & De Jonge, P. (2013). Plasticity genes do not modify associations between physical activity and depressive symptoms. *Health Psychology, 32*(7), 785-792.

Thapar, A., Langley, K., Asherson, P., & Gill, M. (2007). Gene-environment interplay in attention-deficit hyperactivity disorder and the importance of a developmental perspective. *The British Journal of Psychiatry, 190*(1), 1-3.

Van Der Meer, D., Hartman, C.A., Richards, J., Bralten, J.B., Franke, B., Oosterlaan, J., ... Hoekstra, P.J. (2014). The serotonin transporter gene polymorphism 5-HTTLPR moderates the effects of stress on Attention-Deficit/Hyperactivity Disorder. *Journal of Child Psychology and Psychiatry, 55*(12), 1363-1371.

Van Lier, P.A.C., Van Der Ende, J., Koot, H.M., & Verhulst, F.C. (2007). Which better predicts conduct problems? The relationship of trajectories of conduct problems with ODD and ADHD symptoms from childhood into adolescence. *Journal of Child Psychology and Psychiatry, 48*(6), 601-608.

Verhulst, F.C., & Achenbach, T.M. (1995). Empirically based assessment and taxonomy of psychopathology: Cross-cultural applications. A review. *European Child and Adolescent Psychiatry, 4*, 61-76.